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CYSTOMETRICAL EVALUATION OF BLADDER INSTABILITY IN RATS WITH INFRAVESICAL OUTFLOW OBSTRUCTION

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ABSTRACT

Cystometries were performed in normal rats and in rats with bladder hypertrophy due to infravesical outflow obstruction. Investigations were performed in the presence and absence of anesthesia. Pentobarbital anesthesia depressed spontaneous contractile activity in the bladder and the micturition reflex, thereby making measurements of other variables, such as bladder capacity and residual volume, impossible. In conscious animals infravesical outflow obstruction led to development of increased bladder capacity, marked residual volume, and unstable detrusor contractions.

The model seems to be well suited for further evaluation of the mechanisms involved in the development of detrusor instability and the responses to pharmacological treatment.

Infravesical bladder obstruction in man is often associated with detrusor instability and residual urine. The pathophysiological background for the detrusor instability is unknown[1] and also difficult to investigate in man. Animal models of infravesical obstruction have therefore been developed in several species, including the mouse,[2] rat,[3] rabbit,[4,5] dog,[6,7] and pig.[8] To some extent, the models in rat and rabbit have been characterized with regard to bladder morphology and responses to drugs.

Volume-pressure relations and force development in response to pelvic nerve stimulation have previously been recorded in the hypertrophic rat urinary bladder, but only in anesthetized animals.[3] In conscious animals, cystometrical evaluation of the hypertrophied bladder has, to the best of our knowledge, been performed only in obstructed pigs,[8] though this model is less well characterized morphologically and pharmacologically. The aim of the present study was therefore to develop a model for cystometrical evaluation of bladder hypertrophy in the conscious rat.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats were used. Mean weights in control and obstructed groups were 242 gm. (range 185 to 275) and 266 gm. (range 220 to 291), respectively.

Surgical procedures. To obtain a partial obstruction of the urethra, rats were anesthetized with methohexital sodium (Brietal), 70 mg./kg. intraperitoneally. Via a lower midline incision the bladder and the proximal urethra were exposed. A double silk ligature was placed around the urethra and tied in the presence of an intraluminally placed indwelling plastic rod with a diameter of one mm.[3] The abdominal wall was closed and the animals investigated about six weeks after the establishment of obstruction. Bladder outflow obstruction was successfully produced in about 70% of the animals. Acute retention was probably the cause of death in the non-surviving animals.

During anesthesia (sodium pentobarbital 50 mg./kg., intraperitoneally) the abdomen was opened through a midline incision and a polythene catheter (Clay-Adams PE-50) with a cuff was inserted into the bladder. A suture around the catheter closed the bladder incision. In some animals a similar catheter was placed in the abdomen for recording of abdominal pressure thesia, the catheters were tunneled subcutaneously and an orifice was made on the back of the animal. The abdominal incision was sutured and the open ends of the catheters sealed.

Experimental procedures. Cystometry during anesthesia was performed the same day as implantation of the bladder catheter, whereas cystometry without anesthesia was performed the day after. The catheter was connected via a T-tube to a pressure transducer (Statham P23 DC) and an infusion pump (Microinject, Bioinvent). In the anesthetized rat the bladder was emptied and covered with saline soaked cotton wool swabs before the start of cystometry. The conscious rats were placed in a metabolic cage which also enabled measurements of micturition volumes by means of a fluid collector connected to a Grass force displacement transducer, FT 03 C. After an equilibration period the bladder was emptied by means of a syringe connected to the catheter and warm saline solution was infused at a rate of 2.5 ml. $\times$ h^{-1} and 10 ml. $\times$ h^{-1} in control and obstructed rats, respectively. Immediately after a micturition had ceased, the infusion was stopped and possible residual volume measured. The parameters were recorded on a Grass polygraph.

Calculations and statistics. In the anesthetized animals, amplitude and frequency of pressure fluctuations were estimated at a time when bladder pressure had increased by 2.0 cm. H_2O. This point was selected since conscious control rats micturated when the bladder pressure had increased by an average of 2.0 cm. H_2O. In the conscious animals, the period selected for evaluation was two minutes immediately before micturition. In the conscious animals, bladder compliance was calculated as the bladder capacity divided with the pressure increase from start of infusion to start of micturition. Statistical analysis was based on the Student's t test and p values <0.05 were considered to be significant. Data are given as mean values ± SEM.

RESULTS

Partial obstruction of the urethra led to a significant increase in bladder weight ($p < 0.0001$). The mean weight in control rats was 0.139 gm. (n = 12, range 0.05 to 0.28 gm.) whereas the corresponding value in obstructed rats was 0.917 gm. (n = 20, range 0.34 to 2.66 gm.).

Anesthetized rats. Fig. 1A shows a cystometrogram obtained

pressure. Since no micturition occurred during this or any other cystometry performed during anesthesia, no threshold bladder volume for micturition could be calculated.

A cystometrogram obtained in an anesthetized obstructed rat is shown in fig. 1B. The rate of infusion was increased to 10 ml. × h^{-1} to obtain approximately equal infusion times in obstructed and control rats. Pilot experiments showed that an increase in infusion rate from 2.5 to 10 ml. × h^{-1} in obstructed anesthetized rats did not significantly alter the pattern of response. In the obstructed rat small pressure fluctuations were seen during cystometry similar to those observed in the control rat.

Spontaneous activity, expressed as mean amplitude and frequency of the contractions, is shown in fig. 2. As can be seen, there was no significant difference between control and obstructed animals.

Conscious rats. In conscious animals, abdominal pressure was measured by a separate catheter (see Methods). The abdominal pressure was low and only minor fluctuations (<0.75 cm. H_2O) were recorded during spontaneous bladder contractions and micturition.

Fig. 3A shows a cystometrogram obtained in a conscious control rat (a cystometrogram obtained in the same rat during anesthesia is shown in fig. 1A). An infusion rate of 2.5 ml. × h^{-1} generally induced no or only a minor increase in bladder pressure before micturition occurred. The bladder volume immediately prior to micturition (bladder capacity) was in this rat 0.09 ml. and the bladder pressure immediately before micturition (threshold pressure) amounted to 10 cm. H_2O. The measured micturition volume was 0.05 ml. and the residual volume 0.05 ml. As can be seen, there were only small fluctuations in pressure during bladder filling, and their mean frequency and amplitude were calculated to four $min.^{-1}$ and four cm. H_2O, respectively. Maximal bladder pressure during micturition (micturition pressure) was 44 cm. H_2O.

Cystometrograms obtained in conscious obstructed rats showed a more pronounced "spontaneous activity" than those obtained in conscious normal rats. Figures 3B and C show examples of two types of pattern.

The cystometrogram illustrated in fig. 3B was obtained in the same animal as that depicted in fig. 1B. In this obstructed rat, bladder capacity was 9.3 ml. and the threshold pressure 13 cm. H_2O. The micturition volume was measured to only 0.5 ml.; the residual volume was 8.7 ml. The mean amplitude of the spontaneous contractions was 47 cm. H_2O, which was considerably higher than that seen during anesthesia in the same animal (c.f. fig. 1B). The micturition pressure was 216 cm. H_2O.

Figure 3C shows a cystometrogram from an obstructed rat showing more regular spontaneous contractions with lower amplitude than those seen in fig. 3B, although clearly larger than in conscious control rats (fig. 3A). The mean frequency was 2.5 $min.^{-1}$ and the mean amplitude eight cm. H_2O. The bladder capacity in this animal was 2.6 ml. and the threshold pressure amounted to 10 cm. H_2O.

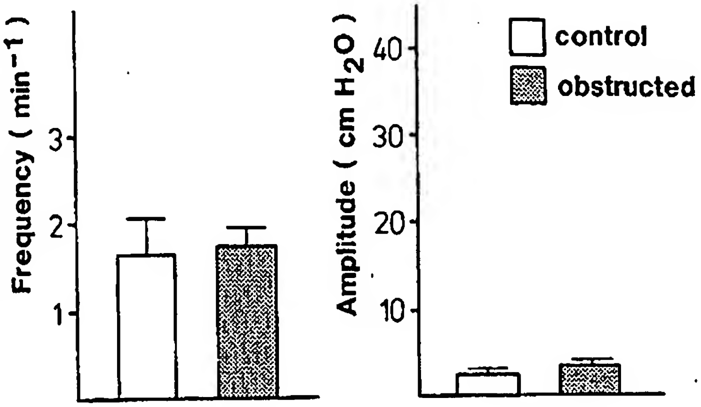


FIG. 2. Collected data on frequency and amplitude of bladder contractions during cystometry in anesthetized control, n = 28, and obstructed, n = 16, animals.

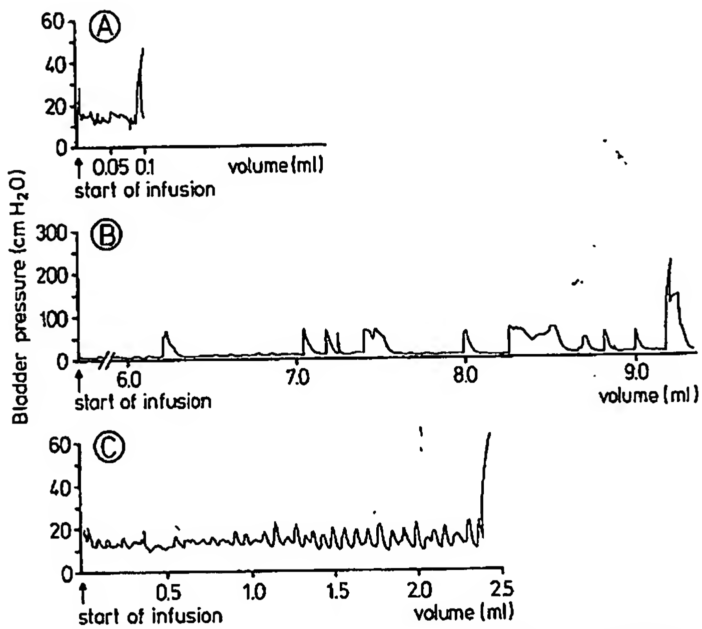
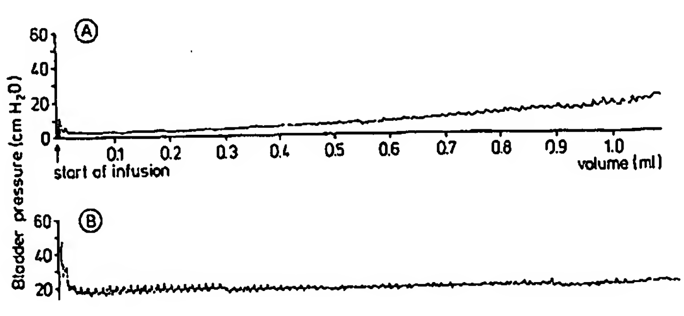


FIG. 3. Original recordings obtained in *A*, conscious control and *B*, *C*, obstructed rats. *A*, control rat (the same animal as in fig. 1*A*). Cystometrograms of *B* and *C* show two different types of patterns obtained in obstructed animals. (Cystometrogram of *B* was obtained in same animal as in fig. 1*B*).

The pressure pattern during a normal micturition cycle in obstructed rats was almost identical to the pattern obtained during infusion. Fig. 4 shows a recording of the bladder pressure during a period without infusion. Early after micturition almost no spontaneous contractions were recorded, but successively spontaneous contractions developed with increasing amplitude until micturition occurred.

The collected data and mean values of the cystometrograms obtained in conscious animals are shown in fig. 5. In control animals the mean bladder capacity (fig. 5A) was 0.16 ± 0.02 ml. and the corresponding value for obstructed rats 4.00 ± 0.86 ml., a more than 25-fold difference (p <0.01). None of the normal animals had a bladder capacity exceeding 0.3 ml., whereas the lowest value obtained in an obstructed rat was 0.8 ml. The threshold pressure was, however, similar in obstructed and normal rats (fig. 5A). A clearcut difference in bladder compliance was noted (fig. 5B), the obstructed rats having a significantly higher compliance than control rats (p <0.01). The mean micturition pressure in control rats was 36 ± 4 cm.



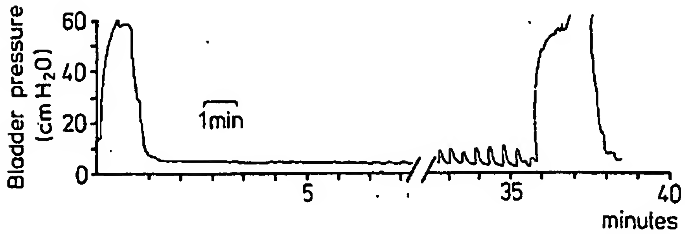


FIG. 4. Bladder pressure recording obtained in obstructed rat during normal micturition cycle, without infusion. Mean frequency of spontaneous contractions recorded during two minute period immediately before micturition was 3.0 $min.^{-1}$ and mean amplitude six cm. H_2O. Micturition volume was 0.7 ml. and micturition pressure 60 cm.H_2O.

difference was noted in the presence and absence of anesthesia, neither in the frequency nor in the amplitude of the contractions (figs. 2 and 5C). The conscious obstructed rats showed a pronounced "spontaneous activity" during cystometry, clearly different from that observed in the presence of anesthesia. In the obstructed rats the mean amplitude of the contractions averaged 23 ± 7 cm. H_2O whereas the corresponding value in control rats was 4 ± 1 cm. H_2O ($p < 0.05$).

As seen in fig. 5D, the micturition volume was clearly lower in the control rats than in the obstructed rats, 0.12 ± 0.02 and 0.65 ± 0.15 ml., respectively ($p < 0.05$). Control rats had a small residual volume (fig. 5D), none of the values exceeding 0.16 ml. (mean value 0.07 ± 0.01 ml.). As a contrast the obstructed rats had a mean residual volume of 3.33 ± 0.88 ml., a 20-fold difference ($p < 0.05$). The lowest value obtained in an obstructed rat was 0.22 ml.

DISCUSSION

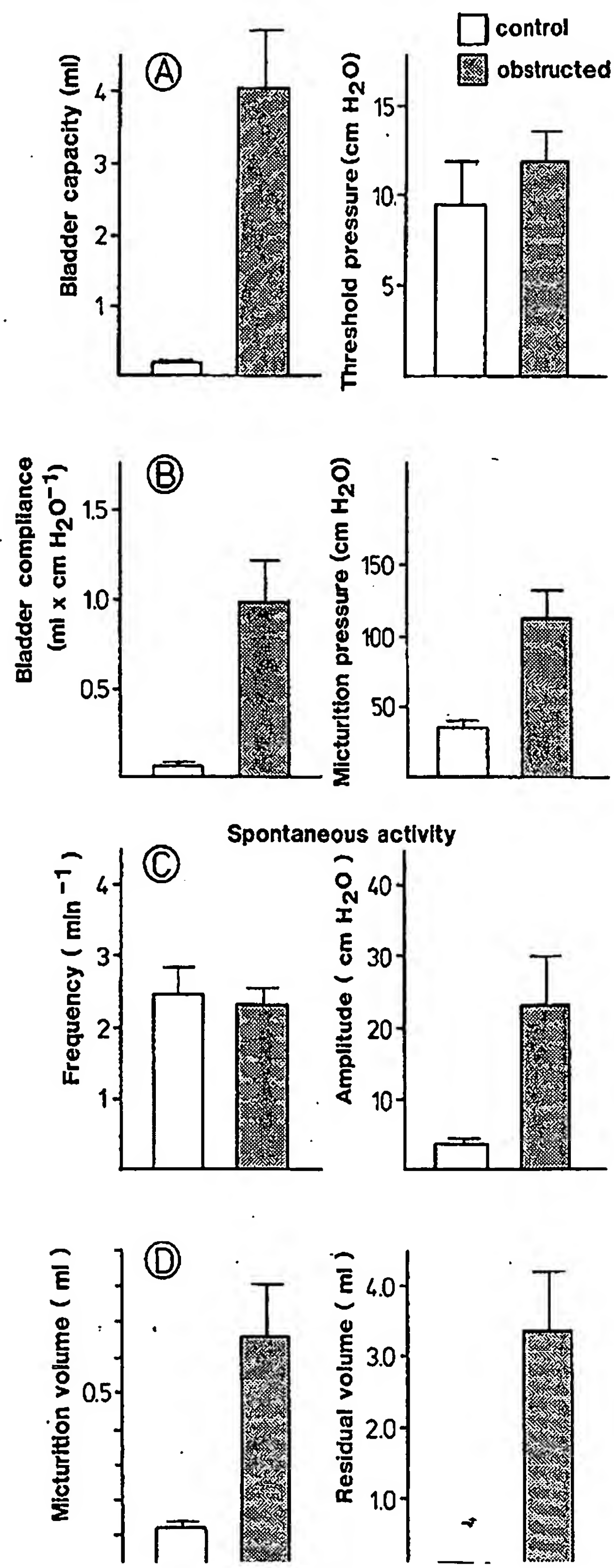
The results of the present study show that infravesical outflow obstruction in rats led to the development of an unstable urinary bladder in the majority of the animals. This condition could be discovered and closely examined by performing cystometries in conscious animals.

In a study on urethane anesthetized normal rats by Maggi and coworkers,[9] an infusion rate of 2.8 ml. × h^{-1} elicited rhythmic contractions of the detrusor muscle assumed to represent the micturition reflex. Such rhythmic contractions were not found to precede micturition in the conscious normal rats in the present investigation, and it is open to doubt whether or not they represent the normal micturition reflex.

During anesthesia small pressure fluctuations were seen during cystometry in both obstructed and control animals, but the amplitude of these contractions could not be used to distinguish between normal and obstructed bladders (figs. 2 and 5C).

The most prominent finding in the cystometries performed in conscious rats was the appearance of spontaneous activity in animals with infravesical outflow obstruction. In man the recommended criterion for bladder instability is a minimum magnitude of pressure rise exceeding 15 cm. H_2O.[10] Using this criterion all but one obstructed rat, or 83%, developed instability, whereas none of the control rats exhibited any contraction exceeding 10 cm. H_2O. A similar high incidence of bladder instability has previously been shown in a model in the pig.[8] In the present model for bladder obstruction there was a good correlation between bladder weight and bladder capacity ($p < 0.01$), but we found no correlation between bladder weight and amplitude of the pressure fluctuations (instability). This is in agreement with observations in man showing little correlation between the severity of obstruction and the incidence of instability.[1]

As discussed by Sibley[8] it is, of course, impossible to know whether the "spontaneous contractions" are voluntary attempts reflect a true detrusor instability. This view is further corroborated by preliminary data showing regular contractions with increasing amplitude during a normal micturition cycle in obstructed rats. Mattiasson and Uvelius[3] have shown that obstructed bladders developed a pressure significantly lower than normal at bladder volumes less than 0.4 ml. If any of the large contractions seen in obstructed animals was voluntary, this might indicate that the obstructed bladder at that particular volume could not develop the force necessary to overcome



the urethral resistance. Such a mechanism might be involved in the development of residual urine.

It might be argued that the spontaneous activity in obstructed rats is caused by the higher infusion rate (10 ml. $\times$ h^{-1}) used in these animals. This seems, however, unlikely for two reasons. Firstly, infusion at this higher rate in control animals did not cause any sign of spontaneous contractions. Secondly, as previously mentioned, spontaneous contractions may occur during the normal micturition cycle in obstructed rats.

In the present investigation in conscious rats, bladder capacity was found to be significantly higher in obstructed than control animals and the former group had a high degree of residual volume. Despite the large volume infused, the cystometrograms obtained in obstructed rats were flat. In fact, the pressure rise was less pronounced in obstructed than in control animals and thus bladder compliance was clearly higher in the former group. This animal model thereby differs from a similar model in the pig,[8] where at least some of the obstructed animals developed a decreased bladder compliance. However, the results in the present rat model agree well with findings in bladder outflow obstruction in man.[11]

Despite the large residual volume in obstructed animals, the functional bladder capacity was higher than in control rats due to a significantly larger micturition volume. The micturition pressure in obstructed rats was significantly higher than in control rats. This is in agreement with findings in man, where outflow obstruction sometimes leads to an increase in micturition pressure.[1]

The present model on conscious obstructed rats is characterized by a high degree of spontaneous activity, residual volume and an increase in bladder capacity and bladder compliance. These characteristics are also found in patients with severe infravesical outflow obstruction. This model therefore seems to be a suitable tool for further evaluation of the mechanisms behind the development of detrusor instability and also for evaluation of responses to pharmacological treatment.

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